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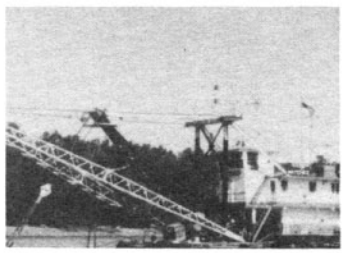

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CHRONIC SUBLETHAL EFFECTS OF SAN FRANCISCO BAY SEDIMENTS ON *NEREIS (NEANTHES) ARENACEODENTATA*; NONTREATMENT FACTORS

by

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13. ABSTRACT *(Maximum 200 words)*

Initial development of a chronic sublethal sediment bioassay with the polychaete *Nereis (Neanthes) arenaceodentata* is described. The test was initiated with 2- to 3-week-old postemergent juvenile worms and terminated after 21 days. The sublethal test end point was estimated individual somatic growth rate (milligrams dry weight/day). The potential bias due to selected nontreatment factors on polychaete survival and growth was evaluated. For example, grain size had no effect, while the number of worms added to each exposure vessel was critical. Direct transfer from 30 ppt to salinities ≤15 ppt had a highly significant and adverse effect on survival and growth. Both survival and growth of juvenile worms may be adversely affected if test conditions involve exposures to ≥20 mg/L ammonia or ≥5.5 mg/L hydrogen sulfide.

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Contents

Preface

The work reported herein was conducted by the US Army Engineer Waterways Experiment Station (WES) for the Headquarters, US Army Corps of Engineers (HQUSACE), and the US Army Engineer District (USAED), San Francisco. Financial support was provided by the USAED, San Francisco, through an Intra-Army Order for Reimbursable Services. Additional funding was provided by the HQUSACE through the Long-Term Effects of Dredging Operations (LEDO) Program, Work Unit 374-9, "Chronic Sublethal Effects." The LEDO Program is managed through the Environmental Effects of Dredging Programs, Dr. R. M. Engler, Manager. Technical Monitors were Mr. David B. Mathis and Dr. William L. Klesch, HQUSACE.

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The work was performed under the general supervision of Dr. Bobby L. Folsom, Acting Chief of the Contaminant Mobility and Regulatory Criteria Group. Mr. Donald L. Robey was Chief, ERSD, and Dr. John Harrison was Chief, EL.

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1 Introduction

Background

San Francisco Bay is a highly altered estuary. Two of the major reasons for this condition are the diversion of freshwater inflow from the Sacramento-San Joaquin River systems and the loss of wetlands. By 1980, the amount of fresh water flowing into San Francisco Bay had been reduced by 60 percent. This reduction is projected to increase an additional 10 percent by the year 2000. About 95 percent of all freshwater/estuarine marshlands had been lost to land reclamation before 1850. It is not surprising, therefore, that the estuary has experienced general decline in health and viability. One of the more noticeable symptoms of this decline has been the gradual loss of biological resources such as the striped bass and Pacific herring fisheries (Nichols et al. 1986).

An increase in the input of environmental contaminants has accompanied the physical alterations of San Francisco Bay. Major pollutant sources include the freshwater inflow from the Sacramento-San Joaquin River systems, over 50 waste treatment plants, and about 200 industries that are permitted to discharge directly into the Bay (Luoma and Phillips 1988). Environmental contaminants discharged into aqueous systems tend to associate with particulate material in the water column and with bedded sediments. Periodically, bedded sediments must be removed to maintain navigable waterways. There is a concern that the relocation of these dredged materials may be causing unacceptable adverse impacts on aquatic biota within the San Francisco Bay.

A large amount of sediment is dredged each year in San Francisco Bay. Approximately 5.5 million cubic meters (mcm) of sediment from Federal projects and permit actions is relocated annually. This value approximates the estimated average annual sediment inflow from natural sources, 6 to 8 mcm (US Army Corps of Engineers (USACE) 1979). It has been estimated that 3 to 4 mcm of material leaves the Bay annually, while Central and North Bays experience a combined net accumulation of 4.2 mcm (USACE 1979). South Bay shows a net loss of nearly 0.8 mcm per year (Krone 1979). Despite these large numbers, the greatest yearly source of suspended sediment in San Francisco Bay is the resuspension of existing

bottom material. Approximately 120 to 130 mcm of sediment is resuspended each year by wind waves and currents (USACE 1979). The effect of these resuspended sediments on fish and aquatic invertebrates is unknown.

To examine whether San Francisco Bay dredged material was causing adverse biological effects, the Planning and Engineering Division of the US Army Engineer District, San Francisco, contracted with the Environmental Laboratory of the Waterways Experiment Station to develop and conduct a series of chronic sublethal sediment bioassays using material from selected sites within the Bay.

Regulatory History of Dredged Material Management in San Francisco Bay

To help define what is known regarding the potential toxicity of San Francisco Bay sediments, it is useful to first examine how dredged material has been regulated in the past. Important milestones in that process are shown in Table 1. It was recognized very early that San Francisco Bay is a physically dynamic system and that most dredged material disposal sites were dispersive. Consequently, initial management concerns were mostly operational. That is, efforts were directed toward optimizing dredging and disposal operations to minimize transportation costs and redredging.

Passage of the National Environmental Policy Act in 1970 outlined the Federal Government's policy toward the environment and signaled an increasing awareness of environmental protection in this country. That same year, the San Francisco District initiated the Dredge Disposal Study (DDS) (USACE 1977). The DDS was a multifaceted interdisciplinary study designed, in part, to address some of the environmental concerns regarding potential impacts of dredge disposal operations. Although sediment toxicity was not examined directly, the physical impacts on biota (USACE 1975b) and the bioaccumulation of contaminants from dredged material were evaluated in laboratory and field studies (USACE 1975c, 1975d). These studies demonstrated that:

a. Estuarine animals can survive suspended sediment loads in excess of those normally encountered during dredging and disposal.

b. In laboratory exposures to San Francisco Bay sediments, estuarine animals can bioaccumulate trace contaminants.

c. In field studies, contaminant tissue concentrations in animals near the disposal operations were not different from those far removed. The one exception was slightly elevated p,p′-DDE concentrations in mussels, *Mytilus edulis*, during disposal. These differences were not detected 1 month postdisposal.

In 1972, the California Regional Water Quality Control Board (RWQCB) adopted the Jensen criteria (Bowden 1977). These numerical criteria were developed by the US Environmental Protection Agency (USEPA) for freshwater sediment in the Great Lakes, and classified sediment as highly polluted, moderately polluted, or slightly polluted based on bulk sediment chemistry. As research on dredged material progressed, it became clear that these and other chemically based numerical criteria were technically inadequate because they did not assess either bioaccumulation potential or toxicity. Both assessments were evaluated in bioassay procedures contained in the USEPA/USACE Ocean Disposal Implementation Manual (USEPA/USACE 1977).

The San Francisco District adopted the use of bioassays for evaluating dredged material. Regulatory procedures were outlined in Public Notice (PN) 78-1. Elutriate procedures were emphasized since disposal sites in San Francisco Bay were generally dispersive. PN 78-1 also reduced the number of disposal sites from 5 to 3. These were located in the Carquinez Strait, San Pablo Bay, and at Alcatraz Island. To facilitate net export out of the Bay, most dredged material was taken to the Alcatraz disposal site.

In 1982, shoaling was noted at the Alcatraz site. As a result of this important development, the San Francisco District took several steps. They instituted a slurry policy to enhance dispersion during disposal. The District greatly reduced the amount of new dredged material taken to the Alcatraz site, and removed 30 tons (27,200 kg) of construction debris from the site. They monitored the physical configuration of the mound at Alcatraz and found it to be stable after two winter seasons. All of these actions led to the conclusion that the Alcatraz site could not be considered fully dispersive. Since the majority of dredged material in San Francisco Bay was taken to Alcatraz, a reduction in the capacity of that site represented a major impediment to maintenance dredging and to anticipated new work activities. The San Francisco District established the Disposal Management Program (DMP) in 1985 and charged it with finding solutions to the disposal problem.

The Long-Term Management Strategy (LTMS) was initiated in 1989 to address increasing environmental concerns and to reflect San Francisco District's commitment to a LTMS for dredged material. In 1991, the Ocean Disposal Implementation Manual was revised to reflect 14 years of regulatory experience and the many scientific advances that had occurred since 1977 (USEPA/USACE 1991).

Overview of Sediment Toxicity Test Development in the United States

As indicated in the foregoing discussion, the regulation of dredged material in San Francisco Bay has taken advantage of scientific advancements that have taken place elsewhere in the United States. To address concerns specific to the potential toxicity of San Francisco Bay sediments it is important to have some general knowledge of advances in the field of sediment ecotoxicology. The following is not intended to be a comprehensive review per se; rather, it is meant to provide the reader a general sense of the advances that have occurred over the past 20 years.

The first peer-reviewed journal article that reported an attempt to assess sediment toxicity was published in 1971 by Gannon and Beeton (Table 2). The laboratory procedures involved exposing amphipods to freshwater dredged material that had been placed in modified milk cartons. In 1973, recognizing the need for a strong technical base in its regulatory program, the USACE initiated the Dredged Material Research Program (DMRP). Included in the scope of this large program was the development of elutriate and solid phase bioassays to assess potential water column and benthic impacts, respectively (Saucier, Calhoun, and Engler 1978). The bioassays developed during the DMRP were subsequently incorporated into both the Ocean Disposal Implementation Manual (USEPA/USACE 1977) and the interim guidance manual for discharge of dredged or fill material into navigable waters (i.e., the 404 Manual) (USACE 1976). These sediment bioassays represented a balance between the state of the art and what could be routinely conducted in a regulatory program.

Prior to the mid-1970s, the scientific community expressed relatively little interest in sediment toxicity. Most of their energies were focused on the fate and effects of environmental contaminants dissolved in aqueous solutions. After the Priority Pollutant List was published in 1976, emphasis shifted for two reasons. One, it was discovered that many chemicals on the Priority Pollutant List were not very water soluble. Second, as more field data were gathered, it became apparent that concentrations of many contaminants on the Priority Pollutant List were much higher in the sediment than in the overlying water. Those findings led to initial speculation that sediments might be extremely toxic. However, subsequent research showed that the same forces causing chemicals to partition into the sediments also restricted their bioavailability to aquatic organisms.

A major milestone marking these scientific advances was the 6th Pellston Conference held in 1984 (Dickson, Maki, and Brungs 1984). This was the first time leaders in the scientific community formally met to discuss the fate and effects of sediment-associated contaminants. Bioassay procedures contained in the 1977 USEPA/USACE Ocean Disposal Implementation Manual formed the basis for initial discussion. The researchers reached consensus regarding sediment toxicity (Anderson et al. 1984). They recognized that species sensitivity was related, in part, to the degree

of contact between sediment and organism. They recommended amphipods and mysid shrimp for lethal tests, and polychaetes, bivalves, oligochaetes, and fish for behavioral or sublethal tests. There was also a strong endorsement of the Tiered Testing Approach for evaluating contaminated sediments (USEPA/USACE 1991). This approach eliminates unnecessary testing and directs limited resources to solving more urgent problems.

Another important milestone in the evolution of sediment toxicity methods occurred in 1987. Members of the American Society for Testing and Materials (ASTM) created a new Subcommittee, E47.01 Sediment Toxicology. This Subcommittee was charged with identifying technically sound procedures for evaluating sediment toxicity and drafting appropriate standardized guideline documents. Guidelines, which are in various states of preparation, include:

a. Solid Phase Toxicity Tests with Freshwater Invertebrates.

b. Solid Phase Toxicity Tests with Marine Amphipods.

c. Solid Phase Toxicity Tests with Marine Polychaetes.

d. Solid Phase Bioaccumulation Tests with Invertebrates.

e. Solid Phase Bioaccumulation Tests with Fish.

f. Guidance for Designing Sediment Toxicity Tests.

g. Guidance for Collection, Storage, Characterization, and Manipulation of Sediment Prior to Toxicity Testing.

When the USEPA/USACE Ocean Disposal Implementation Manual was first published in 1977, the procedures it contained represented a balance between the state of the art and what could be practically achieved in a routine regulatory testing environment. It was realized at that time that revisions would have to be made to reflect anticipated advances in the scientific community as well as experience gained in regulatory testing programs. The Manual was revised in 1991. Significant improvements to the current Manual, as related to sediment toxicity evaluation, include the following:

a. Formalizing the tiered testing approach.

b. Refinements to the species selection process.

c. Provisions for evaluating chronic sublethal effects.

The assessment of chronic sublethal effects is treated as a Tier IV assessment and would be carried out only if there is a reason to believe chronic impacts may be occurring and if technically sound test protocols are available.

Scope

Test procedures for evaluating potential chronic sublethal effects of dredged material on aquatic biota have not been fully developed. Most suggested protocols have been either water column tests ill-adapted for sediment or tests that utilize biological end points with little or no ecological relevance. Before the chronic sublethal effects of San Francisco Bay area sediments can be evaluated in a technically sound manner, a number of issues must be resolved, including (a) identification of appropriate test end points, (b) selection of a test organism, (c) development of test protocol, and (d) development of interpretative guidance.

In acute toxicity tests, only one end point is measured, percent survival. In contrast, a plethora of end points exist for sublethal tests. These end points may be categorized according to the level of biological organization they represent. In order of increasing complexity, these levels are: molecular, cellular, tissue, organismic (whole animal), population, and community. When a sublethal effect occurs at any level of biological organization, mechanistic explanations may generally be found at lower levels while ecological consequences are found at higher levels of complexity.

In the aquatic environment, the ultimate focus of environmental protection is the preservation of viable populations of organisms. Forecasting the potential impact at this level of biological complexity is difficult if not impossible. Bioassessments at lower levels of complexity (molecular-tissue) are possible, but their ecological relevance is uncertain. For these reasons, a surrogate toxicological bioassay approach is desirable. This approach, which examines whole animal (organismic) responses, represents a propitious balance between response sensitivity in the sublethal end point and ecological relevance of the results (Figure 1). Two of the most desirable end points for use in the surrogate toxicological bioassay approach are growth and reproduction. If reproductive success is impaired for a sufficient period of time, the viability of a population may be at risk. In addition, somatic growth and reproductive or gametic growth represent competing energy demands on the bioenergetics of aquatic animals. Therefore, if sediments are shown to reduce somatic growth, reproductive success may also be adversely affected.

Both end points, growth and reproduction, are widely accepted in the scientific community as ecologically relevant. The California RWQCB, for example, has identified growth as a highly desirable sublethal end point. The Board utilizes growth bioassays in its regulatory program for effluent applicants. Test results involving growth and reproduction have the additional benefit of being generally understood and appreciated by a wider nontechnical audience. This latter characteristic is a very important consideration since data for large and/or controversial dredging projects will be carefully scrutinized by the public, and perhaps, the courts.

Selection of an appropriate animal model is another important step in developing a chronic sublethal sediment bioassay. The benthic infaunal polychaete worm *Nereis (Neanthes) arenaceodentata* will be used to evaluate chronic sublethal effects of San Francisco Bay sediments. Several features make this species particularly well suited for use in sediment toxicity tests. First, it maintains intimate contact with the sediment throughout its entire life cycle. Second, unlike many test organisms, *N. arenaceodentata* can be used to evaluate both solid phase and suspended phase material. This allows direct comparisons to be made between the two phases. Third, *N. arenaceodentata* is a sediment ingester. In both solid phase and suspended phase exposures, it readily ingests sediments while foraging for food and tube-building material. Fourth, it is well suited for monitoring of reproductive end points because, unlike most nereid polychaetes, it has no planktonic trochophore larvae. Instead, development is via metatrochophore larvae, which are easier to observe and manipulate from an experimental standpoint. Finally, because the whole life cycle can be completed in the laboratory, cultures producing test organisms of known age and background are possible. This is an attractive logistical characteristic from the perspective of regulatory testing.

Test protocols for a chronic sublethal sediment bioassay with *N. arenaceodentata* have already been developed for the Corps' Seattle District, in cooperation with the State of Washington and Region X of the USEPA. In addition, a "Guide for Conducting Acute and Chronic Sediment Toxicity Test with Polychaetous Annelids" is currently under consideration by ASTM. Both of these tests are 20-day juvenile growth assays initiated with 3-week-old *N. arenaceodentata*.

To have regulatory utility, a sediment bioassay must be able to assess the effects of anthropogenic contaminants in sediment without undue influence from nontreatment factors such as sediment grain size, intraspecific density, and ammonia toxicity. While it is important to evaluate these nontreatment effects in acute toxicity sediment bioassays, it is *critical* to do so for chronic tests. This is because the biological response following chronic exposure is likely to be more subtle (i.e., sublethal) and thus more susceptible to bias from nontreatment influences. For these reasons this report will address important nontreatment effects on survival and growth in the growth assay with juvenile *N. arenaceodentata*. Included will be a discussion of the effects of sediment grain size, intraspecific density, salinity, ammonia toxicity, and tolerance to hypoxia and hydrogen sulfide. The report will conclude with a series of recommendations for conducting chronic sublethal bioassays with the marine polychaete *Neanthes*.

Future reports will focus on interpretative guidance for growth and reproductive end points, bioaccumulation and chronic sublethal effects of bedded and suspended sediments from selected sites in the San Francisco Bay area, direct effects of Bay area sediments on fecundity and larval development, effects of food ration on test end points, effect of storage on sediment toxicity, and a discussion of quality assurance/quality control procedures for chronic sublethal sediment bioassays.

2 Material and Methods

Test Species

Nereis (Neanthes) arenaceodentata is a benthic infaunal polychaete widely distributed in shallow marine and estuarine benthic habitats of Europe, all three coasts of North America, and the Pacific (Reish 1957, 1963; Sanders et al. 1962; Pettibone 1963; Reish and Alosi 1968; Day 1973; Gardiner 1975; Whitlatch 1977; Taylor 1984). This subsurface deposit-feeder constructs one or more mucoid tubes in the upper 2 to 3 cm of sediment and ingests sediment particles up to 70 μ with a preference for particles around 12 μ (Whitlatch 1980). *Nereis (Neanthes) arenaceodentata* has been accepted by the regulatory community as an appropriate test species for evaluating sediment (Johns, Gutjahr-Gobell, and Schauer 1985; USEPA/USACE 1991). A considerable amount of toxicological information on a wide variety of environmental contaminants already exists for this species (Reish 1985, Jenkins and Mason 1988, Anderson et al. 1990).

Taxonomists are still debating the appropriate status of this species. Pettibone (1963), who suggested the name *Nereis (Neanthes) arenaceodentata*, lists five other names for this species: *Spio caudatus, Nereis (Neanthes) caudata, Nereis arenaceodentata, Neanthes cricognatha, and Neanthes caudata*. Day (1973) dismissed *arenaceodentata* in favor of *acuminata*, which was subsequently used by Gardiner (1975), Taylor (1984), and Weinberg et al. (1990). *Neanthes arenaceodentata* is most commonly used in the toxicological literature. Recent evidence suggests that Atlantic and Pacific populations are genetically dissimilar, reproductively isolated, and probably different species (Weinberg et al. 1990). Until the taxonomic status of this species is resolved, we will use the name most familiar to toxicologists and report the original source of worms.

A life cycle of *N. arenaceodentata* is well documented, as are culture methods (Reish 1980). As worms approach sexual maturity, males and females establish pairs and occupy a common tube. Eggs are deposited by the female within the tube. The male presumably fertilizes the eggs at this time. The spent female either exits the tube and dies within 1 to 2 days or is eaten by the male. The male remains in the tube to incubate and

guard the developing eggs. He creates a current of water via rhythmic undulations to remove metabolic wastes and prevent hypoxic conditions.

Larval development is direct via a nonplanktonic metatrochophore larva and occurs entirely within the parental tube. Emergent juveniles (EJs) exit the parental tube about 3 weeks after the egg deposition. They begin to feed and establish tubes of their own. Juvenile worms grow, and eggs become visible in the coelom of females about 6 weeks postemergence. Egg deposition follows 3 to 7 weeks later. The entire life cycle can be completed in the laboratory in 12 to 16 weeks at 20 to 22 °C. The nonplanktonic benthic larva and paternal care are unique among the Nereidae. This feature also facilitates laboratory culture and the experimental investigation of sublethal effects on growth and reproduction.

Laboratory Cultures

Stock populations of *Nereis (Neanthes) arenaceodentata* were obtained in March 1988 from Dr. D. J. Reish, California State University at Long Beach. Laboratory cultures were maintained using methods adapted from those described by Reish (1980) and Pesch and Schauer (1988).

Briefly, the EJs were raised to sexual maturity in 38-L aquaria containing 30 L of 30-ppt seawater (Instant Ocean) maintained at a temperature of 20 °C. The photoperiod was 12 hr light. Animals were fed a combination of ground Tetramarin flakes (2 mg/worm) and alfalfa (1 mg/worm) twice weekly. This feeding regime was sufficient to maintain adequate water quality in a static-renewal system and has been found to produce survival and reproduction consistent with that reported for other laboratory populations of *Neanthes* (i.e., survival >80 percent; fecundity, ca. 100-1,000 eggs/brood; EJ production, ca. 50 to 500 EJs/brood) (Reish 1980, Pesch et al. 1987, Anderson et al. 1990).

Seawater was renewed (80 percent of volume) every 3 weeks. This renewal schedule, based on water-quality monitoring data, was sufficient to maintain good water quality. After 10 weeks, worms were paired using the fighting response (Reish and Alosi 1968) and the presence or absence of eggs in the coelom. Unpaired worms were discarded. Pairs were placed in 600-ml beakers with 500 ml of seawater. Gentle aeration was provided via Pasteur pipettes, and the beakers were covered with watch glasses to reduce evaporation. Water was carefully renewed weekly in such a manner as to avoid disturbing worm pairs.

Beakers were monitored daily for the presence of eggs and EJs. When discovered, EJs are mixed with other broods and returned to the 37-L aquaria to complete the culture cycle. Culture conditions and feeding rations were used in all experiments described below unless otherwise noted.

Nontreatment Factors

Intraspecific densities

EJs were randomly assigned to 600-ml beakers containing 500 ml of seawater at densities of 4.0, 2.0, 1.0, and 0.5X. The value X (two worms per beaker) is equal to the optimal density reported by Pesch et al. (1987) scaled downward to the surface area of the bottom of a 600-ml beaker (ca. 20 cm^2). Food rations were adjusted according to the number of worms per beaker. The experimental regime included five replicate beakers per treatment. Gentle aeration was provided, and seawater was renewed weekly. Survival and growth (established individual dry weights) were determined after 3 to 6 weeks.

The presence of sediment in beakers essentially adds a third dimension (height) to the worms' habitat. Presumably, the number of worms per beakers could be increased. To evaluate this possibility, the above beaker experiment was repeated with 3 cm of the Range Point Pond sediment (90 percent sand treatment, see following paragraph) layered on the bottom of each beaker. There were four replicates per treatment.

Grain size

Emergent juveniles were exposed for 6 weeks to a series of grain sizes created by diluting uncontaminated natural fine-grained sediment with quartz sand that has been combusted in a muffle furnace. Surficial sediment was collected by hand from Range Point Pond, near Pensacola, FL. This site is far removed from any known source of pollution and has been used historically as a control sediment by the USEPA Environmental Research Laboratory at Gulf Breeze, FL (personal communication, Dr. Jim Clarke). The sediment was gently sieved to remove the fine fraction (<0.5 mm). This fraction was proportionally diluted with quartz sand (combusted in a muffle furnace at 500 °C for 12 hr) to yield five grain size treatments, 5, 30, 60, 90, and 100 percent sand. Nominal grain size treatments were confirmed by particle size analysis (Plumb 1981) Sediment was layered in 4-L all-glass aquaria to a depth of about 3 cm. Three liters of seawater was added, and gentle aeration was provided. The following day, 12 EJs were randomly assigned to each aquarium with three replicate aquaria per treatment. Seawater was completely renewed every 3 weeks.

Worms within each replicate were removed after 3 and 6 weeks, counted, briefly rinsed in reverse osmosis (RO) water, and placed on aluminum foil tares. The worms were dried at 60 °C to a constant weight (48 hr), and total dry weight biomass was determined to the nearest 0.01 mg on a Cahn electrobalance. Individual worm dry weights were estimated by dividing the total dry weight biomass by the number of worms in each sample.

Salinity

Three-week-old juvenile worms (reared in 30-ppt salinity seawater) were randomly assigned to 600-ml beakers containing 500 ml of seawater at 30, 25, 20, 15, or 10 ppt. The experimental regime included two worms per beaker and five beakers per treatment. Salinity was determined daily with a hand-held refractometer. Gentle aeration was provided, and seawater was renewed weekly. After 3 weeks the worms were counted, and individual dry weights were determined.

Ammonia

The effects of ammonia (as ammonium chloride) were determined with the same experimental design used to evaluate salinity effects. Preliminary experiments indicated that nominal ammonia concentrations (0, 2.5, 5.0, 10, 20, and 40 mg/L) were very stable under these conditions. During the toxicity test, water samples were taken just prior to each weekly renewal to confirm nominal concentrations. Samples were adjusted to a pH of 2 with concentrated HCl and stored at 4 ^{o}C for no longer than 2 weeks. Total unionized ammonia was determined in this and all experiments with the Orion ammonia-specific electrode after adjusting to a pH of 12 with concentrated NaOH.

Hypoxia and hydrogen sulfide

Hydrogen sulfide occurs in aqueous solution only under hypoxic conditions. Thus, the two factors are inextricably linked. To evaluate the effects of hydrogen sulfide, one must first evaluate hypoxia. A preliminary range-finding experiment indicated that all juvenile worms survived 96 hr at concentrations ≥2.0 mg O_2/L. A definitive test was conducted at nominal oxygen concentrations of 0.25, 0.50, 1.00, 1.50, and 6.50 mg/L. Hypoxia conditions were created by metering high-purity nitrogen gas (99.99 percent) into the normal laboratory air supply. Supply lines were fed through stoppered (two-hole) 1-L Erlenmeyer flasks, each containing 800 ml of 30-ppt seawater. The following day, each flask was briefly unstoppered, dissolved oxygen (DO) was determined (O_2 probe and YSI meter), and five 3-week-old worms were added. There were five replicate flasks per treatment. Temperature, salinity, pH, DO, and survival were recorded on a daily basis, and ammonia was determined at test termination (96 hr). Worms were not fed for the duration of the experiment.

Resistance to hydrogen sulfide was determined using the same experimental protocol as described for the hypoxia experiment. All sulfide exposures were conducted at 1.50 mg O_2/L, the lowest oxygen concentration shown to result in 100-percent worm survival. Each day a stock solution of hydrogen sulfide was prepared by dissolving 60 g of sodium sulfide ($Na_2S{\cdot}9H_2O$) in 1 L of deoxygenated (<0.5 mg O_2/L) RO filtered water. Nominal exposure concentrations (2.5, 5.0, 10, and 20 mg/L) were prepared

from this primary dosing stock. There were two negative controls: normoxia (6.5 mg O_2/L) and hypoxia (1.5 mg O_2/L). Test concentrations were adjusted daily, by adding predetermined amounts of the primary dosing stock to each flask. Hydrogen sulfide concentrations were measured daily (both prior to and following adjustment with primary dosing stock) using a HACH HS-7 test kit. This kit makes use of the reaction between lead acetate and hydrogen sulfide that produces a color change in a lead acetate-impregnated filter pad as PbS is formed. This color change is then compared to a chart to derive a semiquantitative measurement.

Statistical Analysis

Statistical analysis was conducted using the SYSTAT statistical package (Wilkinson 1988). Homogeneity of variance was determined for each biological end point using Bartlett's Test for Homogeneity (Sokal and Rohlf 1981). Dry weights were log-transformed prior to analysis to achieve homogeneous variances. Treatment effects were analyzed using one-way analysis of variance with subsequent mean separation via Tukey's HSD (Honestly Significant Difference) test (Sokal and Rohlf 1981). All tests for significance were conducted at a significance level of $\alpha = 0.05$.

3 Results

Effect of Intraspecific Densities on Juvenile Worms

In the absence of sediment, survival was high (81 to 100 percent) and essentially unaffected at worm densities up to 12 worms/beaker after 3 and 6 weeks exposure (Figure 2). The one exception was significantly lower survival (60 percent) in the highest density treatment after 6 weeks. Growth was unaffected after 3 weeks but was significantly diminished after 6 weeks at worm densities ≥4/beaker (Figure 3).

In the presence of sediment, survival was again high (81 to 100 percent) and unaffected at worms densities up to 12/beaker (Figure 4). Growth was unaffected after 3 weeks exposure, but significantly lower after 6 weeks at worm densities ≥8/beaker (Figure 5).

Effect of Sediment Grain Size on Juvenile Worms

Juvenile worms can tolerate a wide range of grain sizes from 5 to 100 percent sand. Survival was high (89 to 100 percent) after 3 and 6 weeks, and treatments were not significantly different from one another (Figure 6). Likewise, there were no significant effects of grain size on estimated individual worm growth after 3 or 6 weeks (Figure 7).

Effect of Salinity on Juvenile Worms

There was a very sharp threshold response of juvenile worms to salinity. At ≥20 ppt, salinity had no effect on either survival or growth (Figure 8). No worms survived, however, at 15 and 10 ppt.

Effect of Ammonia on Juvenile Worms

As with salinity, there was a very sharp threshold response to ammonia (Figure 9). Survival and growth were unaffected in juvenile worms exposed to 0, 5, and 10 mg/L total ammonia for 3 weeks. Survival was always 100 percent, and mean dry weights ranged from 2.660 to 2.811 mg. Both survival and dry weights were slightly but not significantly diminished (80 percent and 1.840 mg) in worms from the 20-mg/L treatment. Survival was 0 percent in the higher ammonia exposures (40 and 60 mg/L). Ammonia concentrations in the exposure beakers were very stable during the 3-week static-renewal exposures. Prior to weekly renewals, measured ammonia concentrations were very similar to nominal values (Table 3).

Resistance of Juvenile Worms to Hypoxia and Hydrogen Sulfide

There was also a very sharp gradient in the resistance of juvenile worms to short-term (96-hr) hypoxic conditions. Percent survival was 100, 68, and 0 percent in 1.50, 1.00, and 0.50 mg O_2/L, respectively (Table 4). Measured oxygen concentrations were very similar to nominal values. Ammonia was essentially the same in all treatments at the end of the test (ca. 0.20 NH_3 mg/L) except in the very hypoxic treatment 0.25 mg O_2/L (0.07 NH_3 mg/L) where 100-percent worm mortality occurred early during the exposure.

Survival of juvenile worms appears to be unaffected at sulfide concentrations $\leq$5.0 mg/L and adversely affected at concentrations $\geq$10.0 mg/L (Table 5). Mean survival at sulfide concentrations of 2.5, 5.0, 10.0, and 20.0 mg/L were 100, 100, 44, and 0 percent, respectively (Table 5). Measured hydrogen sulfide concentrations generally mirrored nominal values. Measured oxygen concentrations approximated the threshold level where survival of juvenile worms is most affected by hypoxia (0.50 to 1.50 mg O_2/L). This may have obscured the response to sulfide. Also, differences in pH, which varied positively with hydrogen sulfide treatment, may have affected survival in the sulfide treatments. Mean values were 8.52, 8.77, 8.84, 8.87, and 9.42 in the 0-, 2.5-, 5.0-, 10-, and 20-mg/L treatments, respectively.

4 Discussion

High intraspecific densities can adversely affect survival, growth, and reproduction in *N. arenaceodentata* (Pesch et al. 1987). These adverse effects are believed to be the result of more frequent aggressive encounters among worms. This speculation is firmly based in the numerous reports of intraspecific aggression in nereid polychaetes, especially *N. arenaceodentata* (Clark 1959, Evans 1973, Starczak 1984, Kristensen 1988). We have shown that the growth of juvenile *N. arenaceodentata* is unaffected when held for 3 weeks at the same no-effect density reported by Pesch et al. (1987) for nonsediment systems scaled to the bottom surface area of a beaker. The presence of sediment permits slightly higher worm densities. This enhanced survival and growth is probably the result of two factors. Sediment essentially adds a third dimension to the system, depth. As a consequence, the frequency of aggressive encounters is probably diminished. Second, *N. arenaceodentata* is an infaunal burrowing organism with a strong thigmotactic requirement. The presence of sediments is likely to
reduce the general level of biological stress by meeting this requirement even in the absence of intraspecific aggression.

Benthic species often show a preference for specific grain sizes. For example, *Rhepoxynius abronius*, a marine amphipod widely used in sediment bioassays, occurs in well-sorted sand and does not tolerate fine-grained material very well (DeWitt, Ditsworth, and Swartz 1988). Since fine-grained material often carries a higher contaminant load, mortalities in sediment bioassays due to physical impacts in addition to sediment-associated chemicals confound test results and interpretation.

The distribution of sediment-ingesting polychaetes is associated with differences in grain size (Gaston 1987, Kristensen 1988). This distribution reflects not only individual preferences and tolerances but the physical energy of systems as well as available food resources. Results here indicate that survival and growth of *N. arenaceodentata* is unaffected by up to 6 weeks exposure to a wide range of grain sizes (5 to 100 percent sand). McFarland (1981) reported similar laboratory results following 12 days exposure to sediments with various grain sizes. These laboratory findings are consistent with field observations which show that *N. arenaceodentata* inhabits sediments ranging from 2.6 to 87.6 percent silt and clay

(Whitlatch 1977). Thus, for this species, difference in grain size does not appear to be an important bias in sediment bioassays.

Ammonia is highly toxic to many fish and aquatic invertebrates (USEPA 1985). Acutely lethal concentrations (48- to 96-hr LC_{50} values) range between 0.5 and 2.0 mg/L for many aquatic organisms while chronic effects are observed at concentrations generally an order of magnitude lower (Tables 1 and 2 of USEPA 1985). In sediments, ammonia is derived primarily from the hydrolysis of macromolecules and subsequent deamination of amino acids (Santschi et al. 1990). Near the sediment surface, in situ interstitial ammonia concentrations are about 1 mg/L and increase with depth to 20 to 50 mg/L (Ho and Lane 1973; Murray, Grundmanis, and Smethie 1978; Kristensen and Blackburn 1987; Ankley, Katko, and Arthur 1990; Kemp et al. 1990; Lerat, Lasserre, and le Corre 1990).

Jones and Lee (1988) suggested that ammonia may be an important cause of toxicity in marine sediment bioassays. More recently, Ankley, Katko, and Arthur (1990) demonstrated that this was true for freshwater sediments containing substantial amounts of anthropogenic chemicals. If ammonia is a major causative agent in sediment toxicity bioassays, then past (and future) interpretations regarding potential environmental impacts of sediments have been erroneous.

Results reported here for *N. arenaceodentata* suggest that juvenile worms are adversely affected when exposed for 3 weeks to ammonia concentrations ≥10 to 20 mg/L. Fine-grained organic sediments may have interstitial ammonia concentrations that meet or exceed these levels. Another important consideration is the actual in situ exposures for *N. arenaceodentata*. Aller and Yingst (1978) demonstrated that the burrow wall of the deposit-feeding polychaete *Amphitrite ornata* is the site of intense decomposition. Ammonia concentrations were higher at the burrow wall (10 to 20 mg/L) than in the surrounding interstitial waters (1 to 8 mg/L). Irrigation of the worm tube may act to partially offset these elevated ammonia concentrations.

Many benthic animals inhabiting low-oxygen environments have evolved physiologic, biochemical, and behavioral strategies to survive hypoxic conditions (Prosser 1973). While *N. arenaceodentata* can survive low-oxygen tensions, it does appear to have a very sharp threshold response to hypoxia. Davis and Reish (1975) exposed female worms to low DO concentrations for 56 days. Survival and fecundity were unaffected at concentrations as low as 3 mg/L. At 2 mg/L, fecundity was reduced by 50 percent and, at 1 mg/L, both survival and fecundity were severely impacted.

Results reported here indicate that survival of juvenile worms is unaffected under very hypoxic conditions (1.5 mg/L). However, at slightly lower DO concentrations (1.0 and 0.5 mg/L), 96-hr survival drops quickly to 68 and 0 percent, respectively. In sediments, *N. arenaceodentata* avoid hypoxic conditions by drawing water through their tubes via rhythmic undulations. These findings suggest that as long as overlying water is

aerated, hypoxia will probably not be an important nontreatment factor in sediment bioassays with *N. arenaceodentata.*

Hydrogen sulfide is a metabolic poison that is lethal to many fish and invertebrate species at concentrations less that 1 mg/L (Smith, Oseid, and Olson 1976; USEPA 1976; Main and Nelson 1988). Hydrogen sulfide is almost always associated with hypoxic conditions (Theede 1973) since sulfide is rapidly oxidized to SO_4 or elemental sulfur in the presence of oxygen. Thus, H_2S and hypoxic conditions are inextricably linked. This renders laboratory assessments of sulfide toxicity problematic. For *N. arenaceodentata*, this situation is exacerbated by the fact that this species has a very sharp threshold response to declining oxygen tensions. In these experiments, all worms survived sulfide concentrations ≤3.4 mg/L. At slightly higher concentrations (5.5 mg/L), survival was reduced to 44 percent. However, DO concentration in the treatment (1.20 mg/L) approximated hypoxia threshold levels. Consequently, the cause of worm mortalities in this treatment cannot be attributed solely to either H_2S or hypoxia.

Assigning causality to H_2S- or hypoxia-induced lethality from these data becomes less important when one considers in situ sediment exposures and possible detoxification strategies for sulfides. Hydrogen sulfide is produced by the bacterial reduction of sulfates and the putrefaction of proteins. Total sulfides in interstitial water of marine sediments commonly range from 1 to 300 mg/L (Berner 1963; USACE 1975a; Murray, Grundmanis, and Smethie 1978; King et al. 1982; Howarth et al. 1983). In contrast, interstitial H_2S concentrations to which benthic infaunal organisms may be exposed are quite low, from 1 to 30 µg/L in bioturbated sediments to 100 µg/L in undisturbed anoxic sediments (USACE 1975a; McLachlan 1978; Emerson, Jahnke, and Heggie 1984). These relatively low concentrations of interstitial H_2S result from its removal as insoluble sulfides, primarily FeS (Ponnamperuma 1972, Santschi et al. 1990). In addition, some infaunal invertebrates have evolved strategies for excluding or detoxifying H_2S (Powell, Crenshaw, and Rieger 1979; Arp and Childress 1983; Powell and Somero 1983).

It has been shown that some infaunal organisms may even choose sulfidic environments over other habitats (Powell, Crenshaw, and Rieger 1979; Meyers, Powell, and Fossing 1988). Vismann (1990) recently demonstrated that the blood, intestinal wall, and intestinal fluid of two nereid polychaetes *(Nereis (Hediste) diversicolor* and *Nereis (Neanthes) virens)* are capable of detoxifying H_2S via sulfide oxidation.

Based on (a) the low in situ interstitial water concentrations of H_2S, (b) the high survival of *N. arenaceodentata* to parts/million-level H_2S exposures, and (c) sulfide-detoxifying capabilities in nereid polychaetes, H_2S does not appear to be a serious nontreatment factor in chronic sediment bioassays with *N. arenaceodentata.*

A number of critical issues require additional research and resolution. These topics fall under two general categories: methodology and interpretation. An important methodological issue to be resolved is the influence of food ration on chronic sediment bioassays with *N. arenaceodentata.* For example, if adequate nutritious food is furnished during sediment exposures, juvenile worms may feed on this ration preferentially and ignore the sediment as a food source. If inadequate or no food is provided, then sediment ingestion, and thus contaminant exposure, will likely increase. Tests on the same sediment under these two scenarios would probably yield quite different results. Differences in nutritional quality among sediments may also be a significant factor. For example, worms exposed to a nutritionally rich sediment (i.e., high organic content) may assimilate more energy and grow larger than worms exposed to less contaminated (control or reference) sediment with less organic matter. This is not an unreasonable scenario since nutritionally rich fine-grained sediments also often have high contaminant loadings. Taghon and Greene (1990) showed that as the level of enzymatically available protein in sediments declined, the feeding increased in the polychaete *Abarenicola pacifica* until a maximum feeding rate was achieved at 0.05 to 0.1 mg protein/g dry sediment. Below this level, feeding rates declined. However, despite this functional adaptation, growth decreased steadily with decreasing protein concentrations (Taghon and Greene 1990). Clearly, additional research on the influence of food is required.

To have regulatory utility, any chronic sublethal sediment bioassay must be accompanied by technically sound interpretive guidance. For *N. arenaceodentata*, this guidance must be able to answer the question, What diminution in growth is biologically important to *N. arenaceodentata*? For example, if growth in Sediment A is statistically different from Sediment B by 15 percent, is that difference biologically important? What is the minimum required level of absolute growth (milligrams dry weight) or growth rate (milligrams dry weight day^{-1}) for *N. arenaceodentata*? To provide answers to these questions, one must establish the relationship between growth and reproductive success. This relationship must be expressed quantitatively and have predictive value.

5 Conclusions

Conclusions based on this study are summarized below.

- Intraspecific density can have a statistically significant effect on growth and survival in *N. arenaceodentata.*

- The presence of bedded sediment in a test chamber permits slightly higher intraspecific densities than possible in the absence of bedded sediment.

- *Nereis (Neanthes) arenaceodentata* is tolerant of a wide range of sediment grain sizes with no statistically significant effect on either growth or survival.

- *Nereis (Neanthes) arenaceodentata* exhibits a sharp threshold response to salinity with statistically significant effects on survival at salinities ≤15 ppt.

- Juvenile *N. arenaceodentata* can tolerate total ammonia concentrations ≤20 mg/L with no statistically significant effect on either growth or survival.

- Juvenile *N. arenaceodentata* exhibit a sharp threshold response to hypoxia with no statistically significant effect on survival after 96 hr of exposure at DO concentrations ≥1.5 mg/L. Davis and Reish (1975) reported effects on fecundity at DO concentrations ≤3.0 mg/L.

- Juvenile *N. arenaceodentata* tolerate hydrogen sulfide concentrations <3.4 mg/L (DO = 1.5 mg/L) with no statistically significant effect on survival after 96 hr of exposure.

6 Recommendations

Based on the results of this study, the following recommendations are made:

- Tests with *N. arenaceodentata* should be conducted at intraspecific densities ≥25 cm^2/worm (i.e., 2 worms/600-ml beaker) in the absence of bedded sediment, and ≥25 cm^3/worm (i.e., 4 worms/600-ml beaker) with bedded sediment (at a depth of 2 cm).

- Tests with *N. arenaceodentata* should be conducted at salinities ≥20 ppt (overlying water).

- Measured total ammonia levels in tests with *N. arenaceodentata* should be ≤10 mg/L (overlying water).

- Measured DO levels in tests with *N. arenaceodentata* should be ≥3.0 mg/L (overlying water). Note: This level corresponds to levels previously shown (Davis and Reish 1975) to adversely affect reproduction.

- Measured hydrogen sulfide concentrations in tests with N. *arenaceodentata* should be ≤3.0 mg/L (overlying water).

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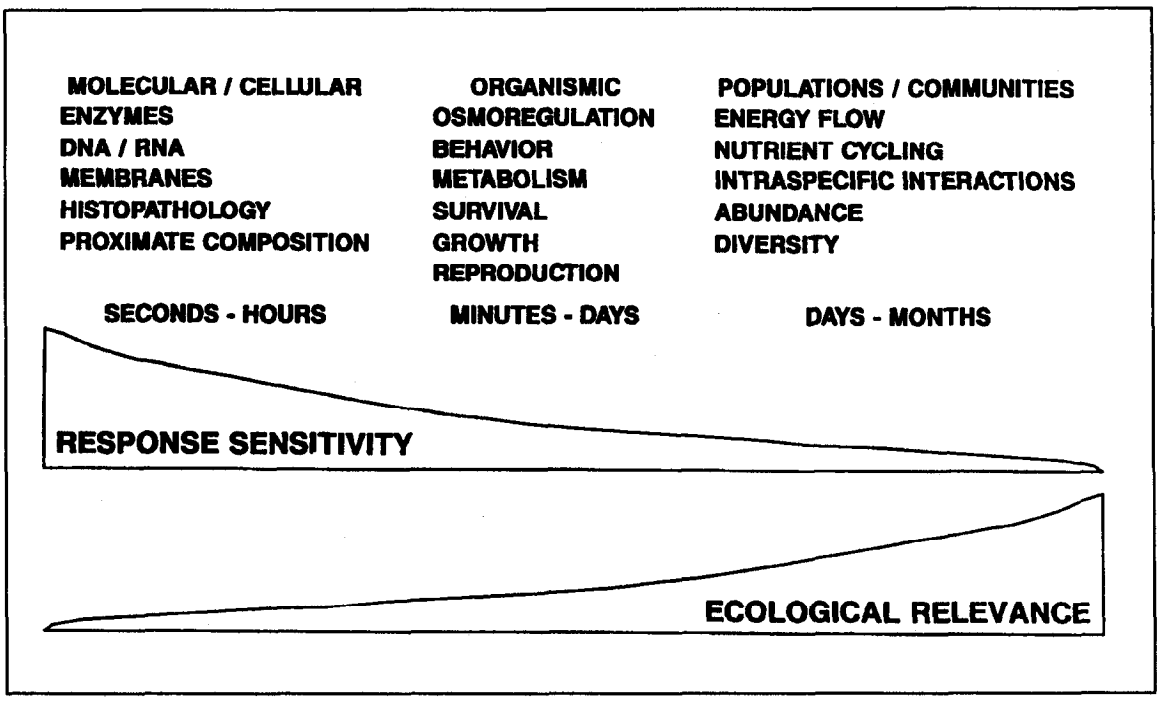

Figure 1. Sublethal end points within levels of biological organization

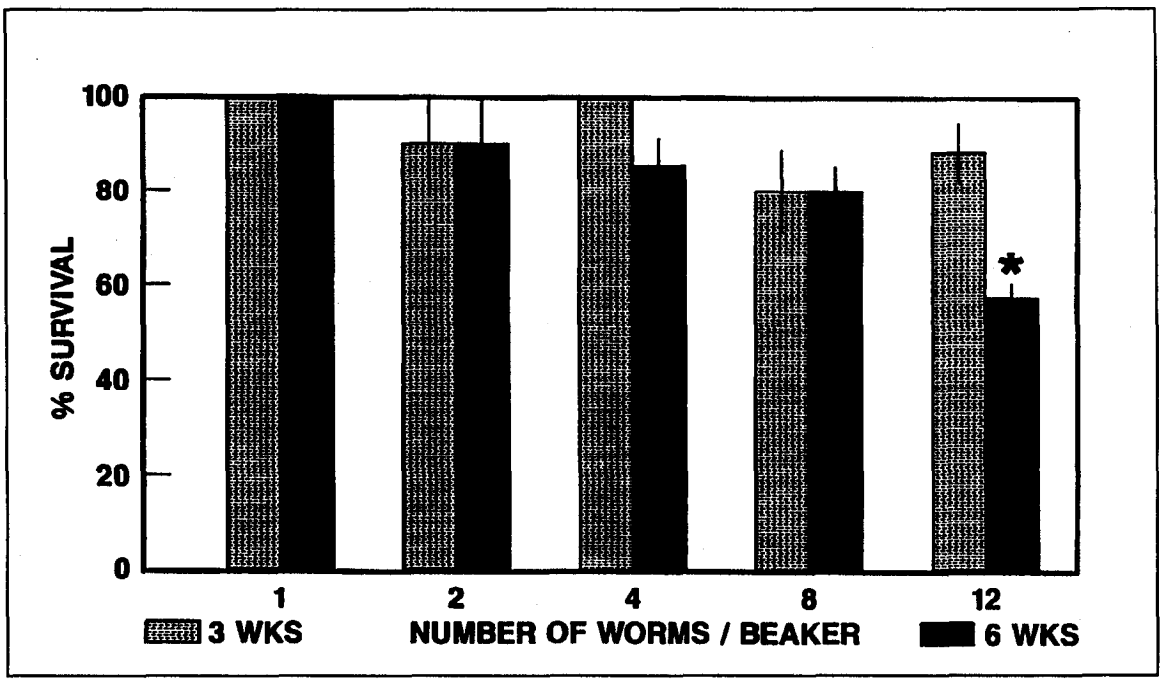

Figure 2. Effect of increasing intraspecific density on mean percent survival of *Nereis (Neanthes) arenaceodentata* in the absence of sediment. Error bars = standard error of the mean. Asterisks = significant difference ($p < 0.05$) from reference 1X treatment (X = 2 worms/beaker)

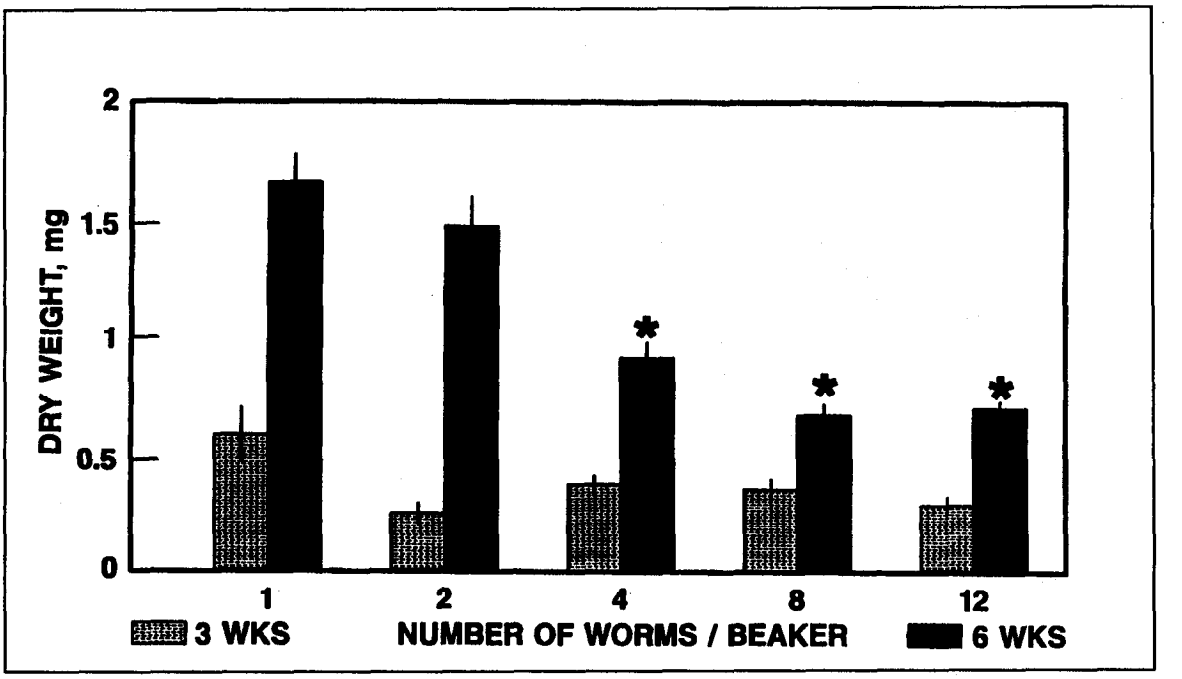

Figure 3. Effect of increasing intraspecific density on mean dry weights of *Nereis (Neanthes) arenaceodentata* in the absence of sediment. Error bars = standard error of the mean. Asterisks = significant difference ($p < 0.05$) from reference 1X treatment (X - 2 worms/beaker)

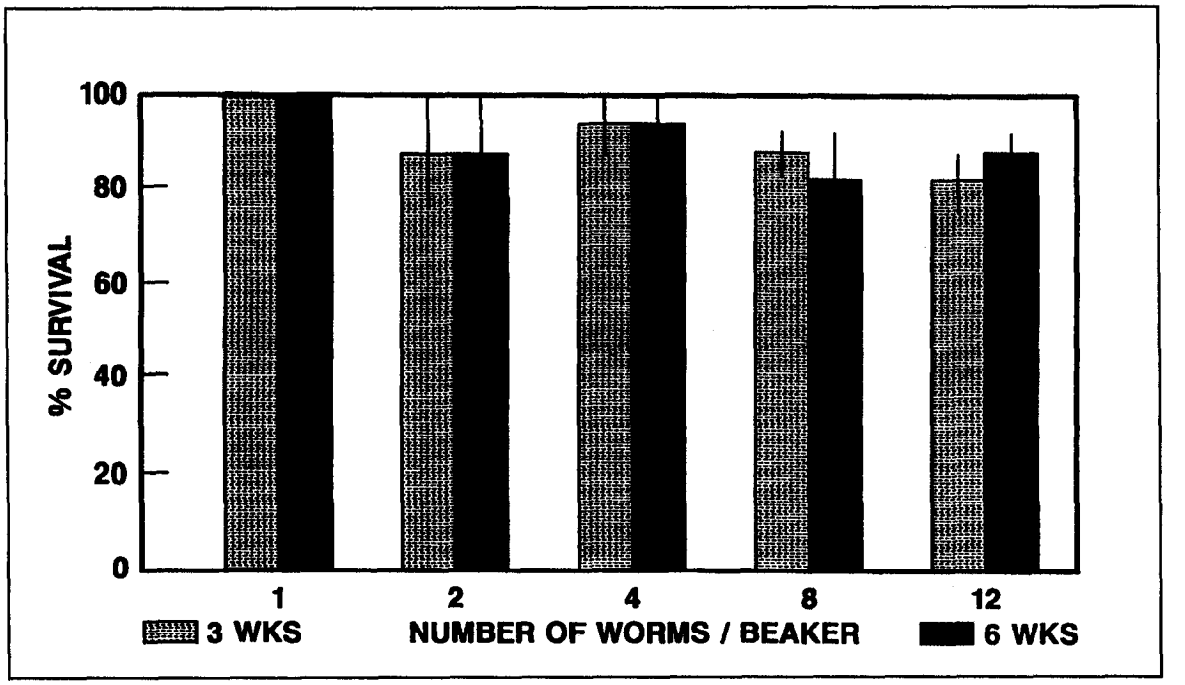

Figure 4. Effect of increasing intraspecific density on mean percent survival of *Nereis (Neanthes) arenaceodentata* in the presence of sediment. Error bars = standard error of the mean

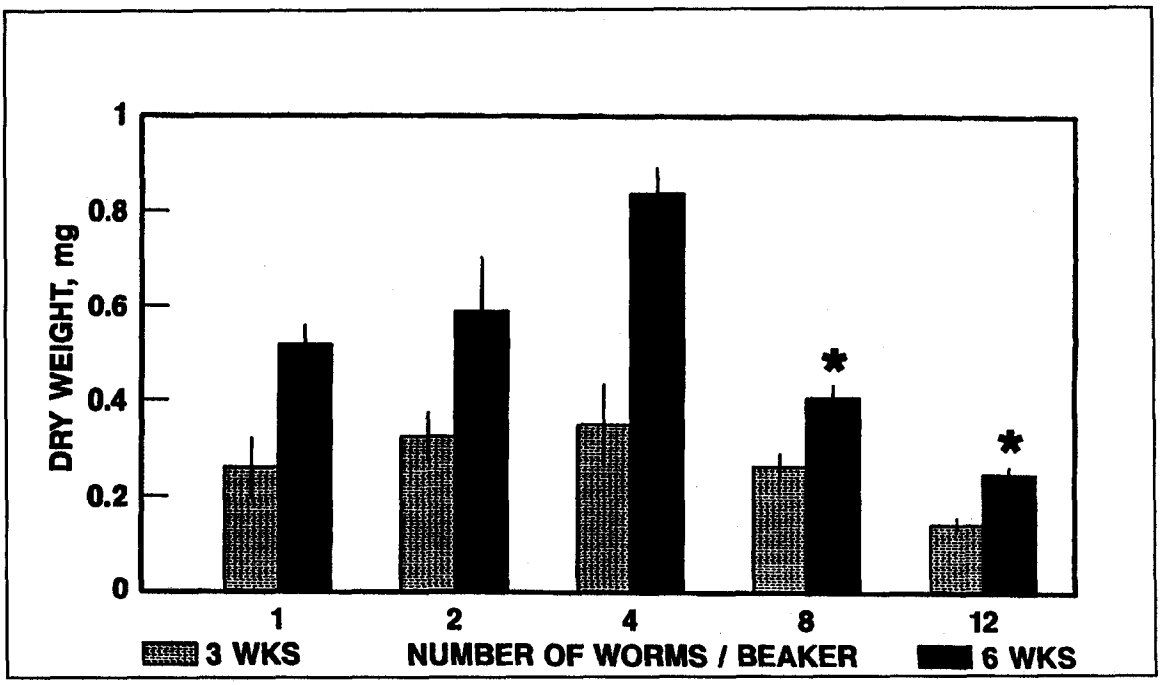

Figure 5. Effect of increasing intraspecific density on mean dry weights of *Nereis (Neanthes) arenaceodentata* in the presence of sediment. Error bars = standard error of the mean. Asterisks = significant difference ($p < 0.05$) from reference 1X treatment (X = 2 worms/beaker)

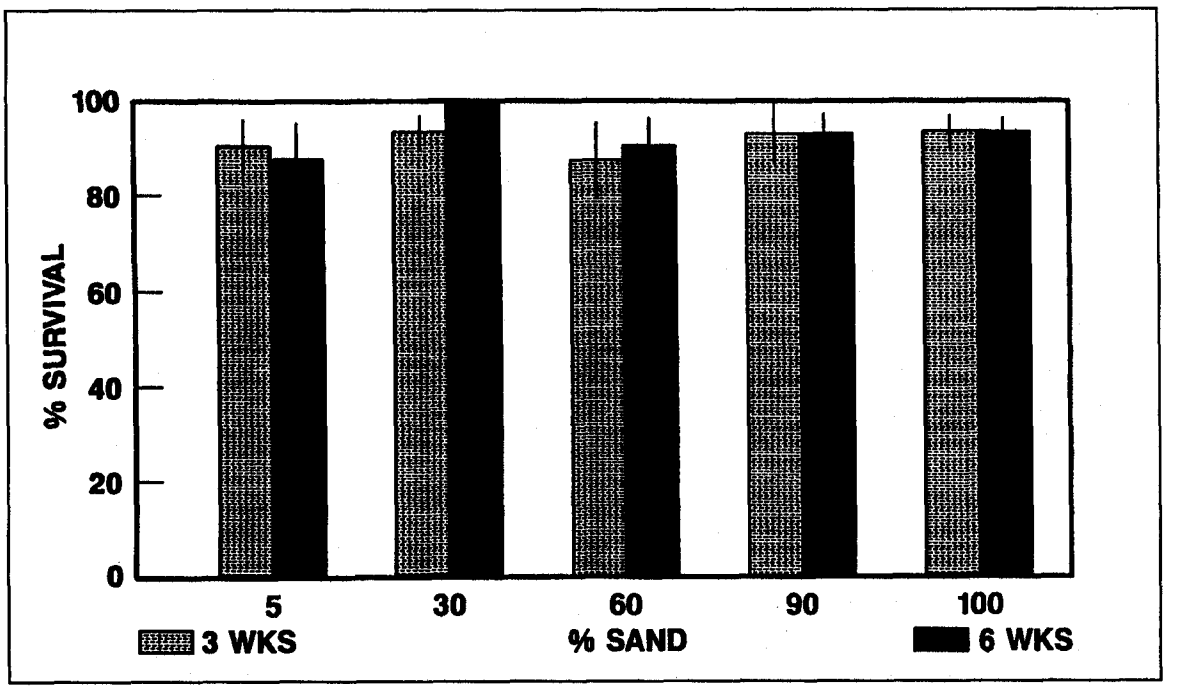

Figure 6. Effect of grain size on mean percent survival of *Nereis (Neanthes) arenaceodentata.* Error bars = standard error of the mean

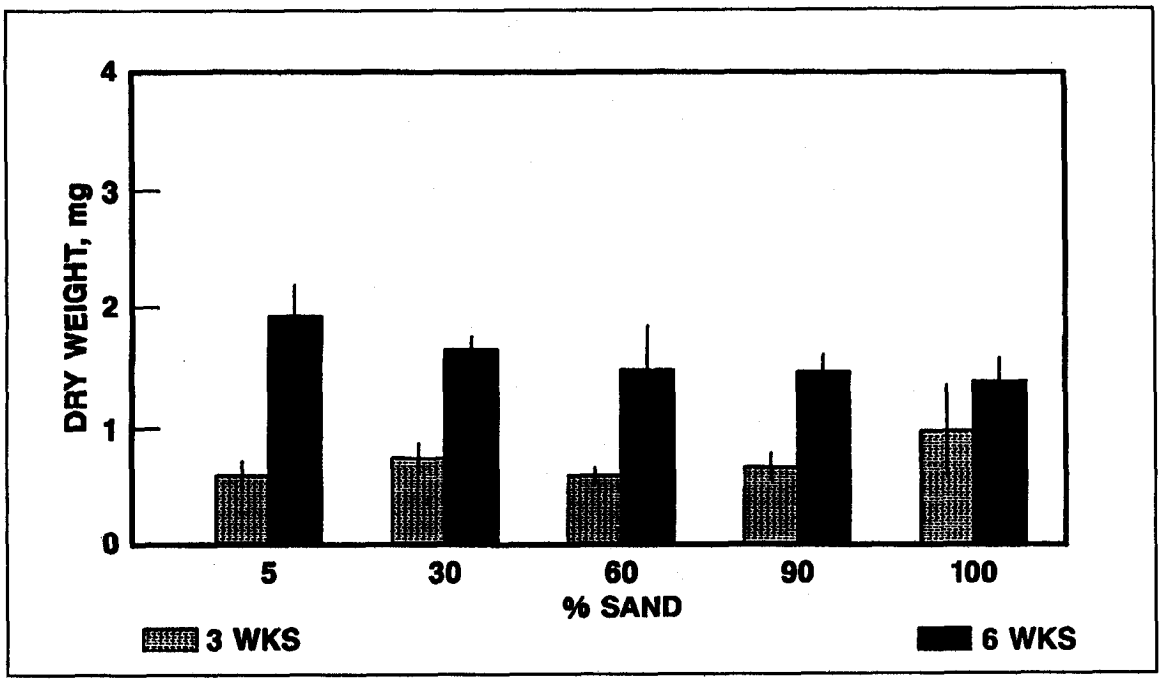

Figure 7. Effect of grain size on mean dry weights of *Nereis (Neanthes) arenaceodentata*. Error bars = standard error of the mean

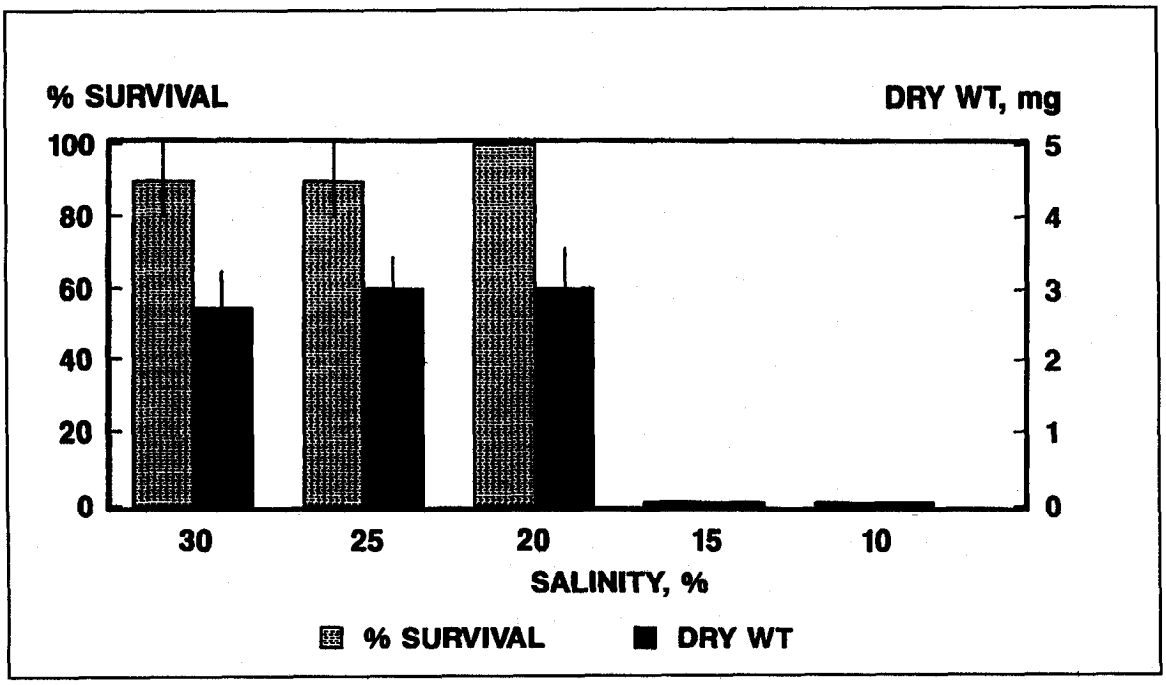

Figure 8. Effect of decreasing salinity on mean percent survival and mean dry weights of *Nereis (Neanthes) arenaceodentata*. Error bars = standard error of the mean

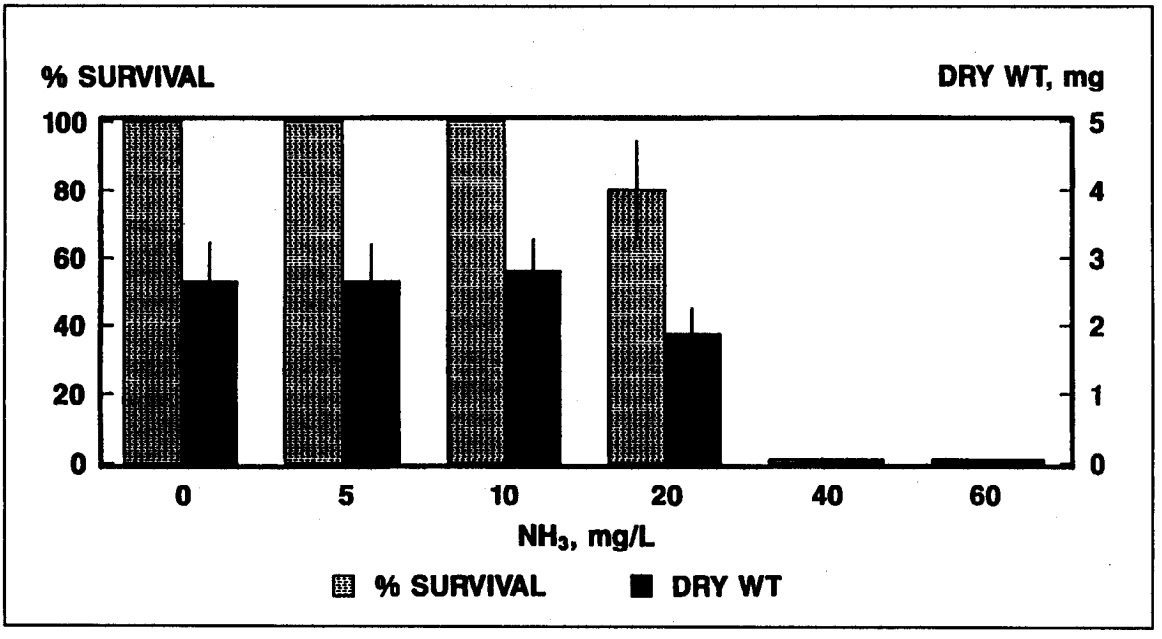

Figure 9. Effect of ammonium chloride (measured as total NH_3 mg/L) on mean percent survival and mean dry weights of *Nereis (Neanthes) arenaceodentata*. Error bars = standard error of the mean

Table 1
Milestones in the Regulation of Dredged Material in San Francisco Bay

1965	Committee on Tidal Hydraulics suggests that San Francisco District (CESPN) may be redredging a significant amount of material.
1970	Passage of National Environmental Policy Act.
1970	CESPN initiates Dredge Disposal Study. Terminated in 1975.
1972	CESPN reduces the number of in-bay disposal sites from 11 to 5.
1972	California RWQCB adopts USEPA's Jensen bulk sediment criteria. Material classified as "polluted" by these criteria was either placed upland or taken offshore to the 180-meter ocean disposal site.
1973	USACE initiates Dredged Material Research Program. Terminated in 1978.
1976	USACE publishes interim guidance manual for implementation of Section 404(b) of Public Law 92-500
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1978	Public Notice (PN) 78-1 issued by CESPN. Elutriate test procedures adopted from the Ocean Disposal Implementation Manual, and in-bay disposal limited to three dispersive sites (Alcatraz, San Pablo Bay, and Carquinez Strait).
1980	California RWQCB adopts PN 78-1.
1980	100-fathom ocean disposal site becomes part of the Point Reyes-Farallon Islands Marine Sanctuary and is subsequently removed from the final designation process by USEPA.
1982	Mounding at the Alcatraz site noted in November.
1984	CESPN implements slurry policy to enhance dispersion during disposal.
1985	CESPN establishes the Disposal Management Program (DMP) to find operational, environmentally acceptable solutions to disposal problems.
1985	San Francisco Bar Channel ocean disposal site receives final designation by USEPA. It can receive only coarse-grained material.
1988	Bioassay procedures in PN 87-1 used to evaluate Inner Oakland Harbor sediments under Section 401 of the Clean Water Act.
1989	The Long-Term Management Strategy was initiated to reflect increasing regulatory and environmental concerns related to dredged material disposal in San Francisco Bay.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

Table 2
Milestones in Scientific Development of Sediment Toxicity Tests

1971	Gannon and Beeton publish first journal article on sediment bioassays.
1973	USACE initiates Dredged Material Research Program. Terminated in 1978.
1976	Publication of Priority Pollutant List by USEPA.
1976	Publication of USACE 404 manual.
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1984	Pellston Conference on Fate and Effect of Sediment-Bound Chemicals.
1987	Formation of ASTM Subcommittee E47.03 on Sediment Toxicology.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

Table 3
Total Ammonia and pH in Beakers Prior to Weekly Renewals During 3-Week Ammonia Toxicity Test

Nominal Concentration mg/L	Week 1		Week 2		Week 3	
	NH_3	pH	NH_3	pH	NH_3	pH
0	0.45[1] (0.04)	8.12 (0.01)	0.79 (0.10)	8.06 (0.04)	0.75 (0.08)	8.07 (0.01)
5	5.32 0.20)	8.10 (0.01)	5.98 (0.13)	8.04 (0.04)	4.54 (0.12)	8.07 (0.01)
10	12.3 (1.83)	8.11 (0.01)	13.0 (1.26)	8.01 (0.04)	13.4 (0.97)	7.99 (0.01)
20	22.4 (0.49)	8.06 (0.05)	19.4 (0.58)	8.02 (0.05)	17.0 (0.32)	8.01 (0.01)
40	41.0 (1.26)	8.05 (0.02)	40.0 (1.67)	7.96 (0.02)	—[2]	
60	66.2 (1.47)	8.04 (0.02)	60.2 (1.60)	7.98 (0.05)	—[2]	

[1] Values represent mean (standard deviation); n = 5.
[2] Treatment terminated because of 100-percent mortality.

Table 4
Water Quality and Percent Survival of Juvenile Worms During 96-hr Exposure to Hypoxic Conditions

Nominal Oxygen Concentration, mg/L	Survival, percent	Dissolved Oxygen mg/L	NH_3, mg/L	pH
6.50 (Controls)	100[1] (0) n = 5	6.64 (0.27) n = 25	0.22 (0.04) n = 5	7.86 (0.19) n = 20
1.50	100 (0) n = 5	1.34 (0.26) n = 25	0.21 (0.08) n = 5	8.18 (0.11) n = 20
1.00	68 (16) n = 5	1.03 (0.19) n = 25	0.21 (0.08) n = 5	8.42 (0.11) n = 20
0.50	0 (0) n = 5	0.50 (0.10) n = 15	0.20 (0.07) n = 5	8.56 (0.07) n = 10
0.25	0 (0) n = 5	0.30 (0.08) n = 11	0.07 (0.03) n = 5	8.69 (0.04) n = 6

[1] Values represent mean (standard deviation).

Table 5
Water Quality and Percent Survival of Juvenile Worms During 96-hr Exposure to Hydrogen Sulfide and Hypoxic Conditions

Nominal H_2S Concentration, mg/L	Survival, percent	H_2S, mg/L	Dissolved Oxygen mg/L	NH_3, mg/L	pH
0.0 (High O_2 control)	88[1] (18) n = 5	0 (0) n = 5	6.59 (0.36) n = 20	0.27 (0.03) n = 5	8.18 (0.03) n = 20
0.0 (Low O_2 control)	52 (23) n = 5	0 (0) n = 5	1.03 (0.16) n = 20	0.24 (0.04) n = 5	8.52 (0.08) n = 20
2.5	100 (0) n = 5	1.4 (1.3) n = 5	1.33 (0.12) n = 20	0.32 (0.03) n = 5	8.77 (0.05) n = 20
5.0	100 (0) n = 5	3.4 (1.4) n = 5	1.74 (0.19) n = 15	0.24 (0.07) n = 5	8.84 (0.06) n = 20
10.0	44 (30) n = 5	5.5 (2.9) n = 4	1.20 (0.31) n = 20	0.16 (0.09) n = 5	8.87 (0.34) n = 20
20.0	0 (0) n = 5	15.0 (4.1) n = 3	0.96 (0.12) n = 15	0.45 (0.08) n = 5	9.42 (0.20) n = 15

[1] Values represent mean (standard deviation).

Waterways Experiment Station Cataloging-in-Publication Data

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Includes bibliographical references.
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